Down-regulation of CK17 Inhibits Cell Proliferation and EMT in Gastric Cancer Cells

Kaifeng Hu[a], Yabin Xia[a], Hao Hu[a], Yan Jin[a], Jiebin Wu[*b]

Abstract

Cytokeratin 17 (CK17) is an epithelial basal cell / muscle keratin released by the activated keratinocytes. It is not present in most somatic cells, but in some stem cells and some malignant tumors in cervical cancer, ovarian cancer, head, and neck tumors, etc. A systematic research on the control and function of CK17 in gastric cancer is not performed recently. The study explored CK17 regulatory mechanism for the spread, apoptosis, resettlement, incursion of gastric cancer cells. RT-PCR, Western blot detected mRNA and CK17 protein concentrations in 72 gastric cancer nerves and 5 gastric cancer cells. A link in the middle of CK17 representation and clinical pathological parameters and prognosis had been studied within diseased person's patients with gastric cancer. Lentivirus has been leading down-regulation of CK17 in gastric cancer cells. CCK-8 detected cell spreading assay and Tran's well detected plate cloning assay. Cell migration and invasiveness. Manifestation of EMT-connected proteins within gastric cancer cells was calculated using Western blot. CK17 expression within gastric cancer nerves and cell areas was substantially increased, its level of representation closely linked to stage TNM, lymph node metastases within patients with gastric cancer. Prognostic studies found the diseased persons having minimal manifestation of CK17 possessed a longer period of subsistence concerning gastric cancer. Cell experimental findings showed the down-regulation of CK17 prevented the spread, resettlement, penetration about gastric cancer cells. Levels of expression of N-cadherin and Vimentin proteins have been substantially reduced, however appearance of E-cadherin and Δ-catenin has increased significantly. CK17 is highly expressed within gastric cancer and is thoroughly associated with phase TNM, lymph connection metastases and prognosis. CK17 can promote propagation, resettlement, incursion, EMT of gastric cancer cells and inhibit apoptosis of cells.

Key words: gastric cancer; CK17; cell proliferation; EMT.

1. Introduction

Gastric cancer seems broadly recognized harmful gastrointestinal cancer and possesses high morbidity and mortality rate worldwide that affects human health seriously.

The frequency of gastric tumor diminished within developing countries more than previous 20 years, although the mortality rate persists at highest of all malignant tumors. The environmental or genetic considerations like eating forms, chronic atrophic gastritis, Helicobacter pylori infection, etc. influence the prevalence of gastric tumor. Between them, the WHO has listed Helicobacter pylori being the category I carcinogen[1~2]. Actually the

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recommended cure towards abdominal tumor is clinical resection followed by chemotherapy. The clinical diagnosis rate of early gastric cancer has been increased with the ongoing advancement of endoscopic procedures and modern biodiagnostic methods, but most patients with late-stage gastric cancer are either diagnosed or tumor cells have metastasised. As a result, the ideal surgical time is skipped, rendering a bad prognosis for patients with stomach cancer. For the growth of lengthy-tenure subsistence ratio and quality about life of diseased persons with gastric cancer, search for new early indications and therapeutic targets for gastric cancer is of great importance. The advancement of molecular biology and gene technology in recent ages has provided new insights and opportunities for studying the molecular mechanism of gastrointestinal tumor. Targeted medication for gastrointestinal tumor is accomplished by controlling expression about different genes, and has become a focal point for research into gastric cancer.

Cytokeratin (CK) is an crucial component of the cytoskeletal fibers which make up epithelial monolayer cells. It is the representative of intermediary group of fibroids, conveyed mainly in epithelial tissue but small in most mature cells[3]. It has been shown in recent years that CK has stable and high expression in a variety of malignant tumors, especially epithelial-derived malignant tumors, that differential expression is expressively linked to the degree and form of tissue source and the degree of cell differentiation, and is concerned within regulating cancer cell immigration, incursion and drug resistance[4]. CK17 is a large molecular keratin used to treat malignant swellings like lung cancer, oral squamous cell carcinoma, cervical tumor clinically. CK17 can control the tumor suppressor protein p27 expression, promote cell progression progression, hence function like an anti-tumor factor in the incidence and progression of various malignant cancers. Recent studies have shown that CK17 is up-regulated in tumors such as cervical cancer[5], thyroid cancer[6], breast cancer[7], and nasopharyngeal carcinoma[8], with tumor proliferation, migration, invasion, and angiogenesis involvement. The level of expression affects cancer patients' prognosis but expression and functional studies of CK17 within gastrointestinal tumor is rarely published.

The conducted research highlights the appearance of CK17 within gastrointestinal tumor tissues was observed, the suggestion concerning appearance of CK17 and clinicalopathological parameters and prediction about diseased persons through gastrointestinal tumor was analyzed such that the clinical significance of CK17 in the ailment was initially explored. Additionally, CK17 's stable overexpressing or silencing of gastrointestinal tumor cell connections were identified. Roles towards CK17 within regulating cell propagation, apoptosis, invasion, migration were analyzed and the effect of CK17 within gastrointestinal tumor cells on the appearance of EMT-related proteins had been investigated. The functions of the CK17 system were studied in advance.

2. Materials and procedures

Patient knowledge

Selection of 72 patients with gastric cancer diagnosed in our hospital between 2013 and 2014, including 44 males and 28 females aged 39-70 years, with an average age of 57.25±1.06. Both patients underwent radical resection to acquire fresh tumor tissue and samples of normal tissue adjacent to it. The typical neighboring tissues obtained from the lesion were more than 5 cm away. The specimen was immediately placed into liquid nitrogen and then moved for storage to a-80 ° C refrigerator. Prior to surgery, patients received no anti-tumor treatment, and two senior pathologists received confirmed gastric cancer specimens. Patients and their relatives have consented informedly and got permission letter from authority . The ethics panel of the medical Centre approved the Recent work.

Cell lines and the culture of cells

Medical educational Institution of China named Basic Medical Sciences of Medicinal Sciences gained five gastrointestinal tumor cells (AGS, SGC-7901, MGC-803) and perfectly standard gastric mucosal cells (GES-1). All cells had been grown within a DMEM medium holding 10%, 37 ° C culture, 5% CO_2 cell incubator.

Immunohistochemical Staining

Immunohistochemistry staining was conducted using the two-step Max Vision process and was carried out in compliance with the manufacturer's instructions. Tissue samples have been deparaffinized and washed with water, incubated with a solution for antigen recovery. In the cells, andogenous peroxidase was blocked for 10 min by 3 percent hydrogen peroxide. The goat serum was first applied and blocked for 15 min, then the primary

antibody CK17 (diluted 1:1000) was added dropwise to the parts of the tissue and incubated overnight at 4 ° C. At room temperature the secondary antibody was applied dropwise for 15 min to the room temperature. DAB was developed for 1-2 min, and hematoxyline was used to countertain. Dehydrated and clear operations were done, and neutral rubber sealed the slides. The digital section scanner from Japanese Hamamatsu scanned all of the sections into microscopic images. Score system for positive cell expression of CK17: it has been shown that the staining strength of gastric tumor cells is weak, medium and high .It was graded by keeping in view the basis of proportion of optimistic cells in proportion of all tumor cells: < 5 per cent had been graded as 0.5-25 per cent as 1 point, 26-50 per cent as 2 points, 51-75 per cent as 3 points and > 75 per cent as 4 points. The result was derived from the bruising concentration record and the proportion of encouraging cells in all tumor cells and multiplied to produce the immunoreactivity score (IS). The IS was deemed negative by 0 communautaire<3, and positive by 3 < IS<12.

TR-PCR

Trizol processed the complete tumor tissue and cell RNA, and transcribed the cDNA in the reverse transcription pack. The procedure was done as per the instructions in the reverse transcription package. The RT-PCR was executed using cDNA as a guide using the SYBR Green process. GAPDH has been used as an internal control tool. The program Primer 5.0 was used to construct the respective primers. The PCR prime sequence was: CK17 upstream first sequence 5'-TTA GTC GAT TCG CGA TTG GA-3', downstream first sequence 5'-CGA GCT AGC ATG CAT GCA TCC TA-3', GAPDH upstream first sequence 5'-CGG ATG CTA CAA GCT CT-3', downstream first sequence 5'ACG CAG GGT AGC ACT ACG ACG ACG AC-3'. The PCR cycle profile was described as pre-denaturation for 30 s at 94 ° C; denaturation for 30 s at 94 ° C; annealing for 30 s at 58 ° C; extra time for 2 min at 72 ° C; and 35 cycles. The target gene mRNA 's comparative expression level was determined using the 2- aller process.

Cell proliferation Assay

During the logarithmic growth process, cells were accumulated in each transfection group, and digested with trypsin to prepare for cell suspension. Cells had been planted to a 96-well plate (100 μl / well) with the thickness of 5 prob103 cells / well, and 10 μl was added to each well. For another 2 hours the CCK-8 reagent was placed in an incubator with carbon dioxide. A microplate reader measured the absorbance values of the respective wells at different times at a wavelength of 450 nm, and three replicates were determined for each sample.

Migration and Invasion Assay

Cell migration assay: cells with good growth status were trypsinized and resuspended to prepare the separate cell interruption in a serum-free method after transfection24. The cell concentration was changed to 5 ubiquitous105 cells / ml and the cell suspension was applied to the Matrigel along the chamber sidewall. 600 μl of RPMI-1640 method, covering 10% FBS, had been put in smaller space compartment and cultivated in an incubator of carbon dioxide at 37 ° C, 5% CO 2 on 16 h. After washing cells thrice for 5 minutes by PBS buffer, fixed for 30 min with polymethanol, rinsed thrice by PBS, stained for 30 min with 0.05 per cent violet crystal, and washed 3 times by PBS. The membrane had been stripped off, and a neutral resin was sealed. Cells which could not move through the higher side of chamber had been washed off. Cells were dried at the surface, and 5 sights were randomly chosen to be photographed and counted under the microscope.

Cell invasion assay: a mixture of the matrigel and pre-cooled serum-free RPMI-1640 medium at 1:8 ratio. The ice surgery was on. 100 μl Matrigel was gradually increased the Transwell container, incubated for 1 hour at 37 ° C. Gel got solidified. The other steps are in keeping with the migration experiment.

Western blot

The cells with good growth status were taken after 48 hours of transfection, and overall protein from each group had been extricated with RIPA lysis reagent. The protein has been quantified by process BCA. SDS-PAGE electrophoresis was performed with a protein charge of 30 μg per lane and transferred to PVDF membrane. The membrane had been stopped for 90 minutes 5 per cent skim milk powder. Add 1:1000 distinctive primary antibody anti-CK17 and 1:20000 diluted primary antibody anti-GAPDH to the membrane, keep warm for the night on 4 ° C. Rinse the membrane by TBST 3 times every time for 10 min. Eliminate the secondary antibody (anti-rabbit goat) labelled with horseradish peroxidase. The membrane was rinsed 3 times through TBST after incubating at temperature of room for 1 h. DAB developed into a dark space. Photos were obtained using a gel imaging system, and used Quantity One software for analysis of the gray scale.

Statistic Evaluation

Whole statistics is articulated as standard mean ± deviation (x ± s). Analysis of data was carried out using SPSS 22.0 software. T-test had been applied to compare the double classes, and assessment of the difference had been utilized to compare different groups. The parameters of substantial difference is P<0.05.

3. Results

Taken, CK17 extraction and the association concerning clinicalopathological factors within gastrointestinal tumor.

Immunohistochemical tests showed that 47 cases of samples of gastric cancer tissue were positive for CK17 protein with a positive expression rate of 65.28%, while only 7 cases were positive for adjacent typical tissue with a positive expression rate of 9.72% (Fig. 1A, Fig. 1B). The difference (P < 0.05) is numerically important. Outcomes of RT-CPR showed that the CK17 mRNA degree within gastrointestinal tumor tissues had been considerably elevated as compared to adjoining natural muscles (Fig . 1C), consistent with the results of immunohistochemistry. In addition, clinicalopathological parameters were examined for the association between CK17 expression and diseased persons concerning gastrointestinal cancer. Results showed that CK17 in diseased persons with gastric cancer, regardless of gender , age, degree of differentiation, and tumor size, was strongly connected through TNM step and lymph point metastases (e.g. Table 1).

Correlation analysis between CK17 and prognosis of gastric cancer patients

Kaplan-Meier has performed a correlation study between CK17 expression and general persistence (OS), illness-unrestricted persistence (DFS) for establishing medical importance of CK17 extraction concerning predicting prognosis of diseased persons along with gastrointestinal tumor. The results showed that CK17 's high expression was closely connected with the poor prognosis of diseased persons concerning gastrointestinal cancer. Diseased persons with minimal CK17 representation had extensive OS , RFS as compared to the diseased persons along with high CK17 extraction (P<0.05), as shown in Fig. It is from 2A & 2B. Furthermore, a univariate and multivariate regression analysis on the clinical data of patients with gastric cancer was conducted applying the COX degeneration standard.

The findings indicated that expression of CK17, presenting of TNM and metastasizing of the lymph node were independent risk factors for diseased persons including gastrointestinal tumor (P<0.05), as shown in figure 2.

CK17 expression in gastric cancer cell lines

RT-PCR has discovered extraction of CK17 mRNA in 5 cell lines. The findings demonstrated that demonstration of CK17 mRNA in five lines of gastrointestinal tumor cells had been considerably greater compared to GES-1 cells. The most noticeable among these was the up-regulation of CK17 mRNA level within SGC-7901 cells (Fig. 3A). And we picked SGC-7901 for the testings below. While Western blot findings were identical to mRNA findings. CK17 protein representation had been significantly greater within gastrointestinal tumor cell lines compared to usual GES-1 gastrointestinal mucosal cells (Fig. 3B). In gastric cancer CK17 has also been shown to be up-regulated.

Establishment of a CK17 SGC-7901 Stable Silencing Cell

Further exploring the influence of CK17 at biological purpose about abdominal tumor cells, siRNA-CK17 and its siRNA-con negative control had been transfected into cells of SGC-7901, and Western blots were detected. The protein representation degree of CK17 had been substantially lower within the siRNA-CK17 group compared to negative monitor unit, the disparity had been also numerically considerable (P<0.05), as shown in Fig. 4. The results showed the transfection had been successful.

Effect of CK17 on cell proliferation of gastric cancer cells

By describing effect of CK17 at gastrointestinal tumor cell proliferation, a CCK-8 assay had been first identified for cell feasibility of each community of gastrointestinal tumor cells. Findings showed that the viability of the siRNA-CK17 cells was noticeably smaller compared to power unit (P<0.05), as shown in table. 5. Cell cluster pattern test outcomes showed that amount of clones within the siRNA-CK17 unit (202.7±8.6) had been considerably smaller compared to siRNA-con adverse monitor company (402.1±13.9) (P<0.05). It indicated CK17's silencing expression may inhibit its proliferating potential.

Effect of CK17 on cell migration and on invasion of gastric cancer cells

The Transswell migration assay outcomes demonstrated that amount of cells in the siRNA-CK17 group had been considerably as compared to siRNA-controll unit (P<0.05). We found a similar result in the Transwell invasion assay to the cell migration assay that the number of invasive gastric cancer cells decreased markedly by silencing CK17 (P < 0.05), as shown in Fig. 6. CK17 has been shown to be efficient of impeding the invasive capability of gastric cancer cells.

CK17 prevents EMT in gastric cancer processes

Western blots have been used for identification manifestation of EMT-associated indicators within gastrointestinal tumor cells in order to assess if EMT is involved within the EMT cycle. Outcomes demonstrated that degrees of representation within siRNA-CK17 cells had been considerably smaller than within the siRNA-control unit, but points of representation in E-cadherin were significantly higher than in the siRNA-con group (P<0.05), as shown in Table. 7, which indicates that CK17 is capable of promoting the gastric cancer EMT cycle.

4. Discussion

The pathogenesis of gastric cancer is complex , involving multiple proto-oncogenes and tumor suppressor genes being misregulated. Up-regulated proto-oncogenes in tumors generally work the position within promoting tumorigenesis, evolution, like p53, PTEN, etc., while tumor suppression genes are down-regulated in tumors and do the job within inhibiting tumor cell production or in promoting apoptosis, such as heat shock proteins (HSPs), β-catenin, etc.[9~10]. Cytokeratin (CK) is a cytoskeleton-type intermediate protein. More than 50 types of cytokeratin have been identified and can be expressed in various types of epithelial cells[11]. CK is strongly conserved and tissue-specific, and is closely linked to practices of cell proliferation and epithelial keratinocyte differentiation. Additionally, CK portrays vital position concerning maintaining morphology about epithelial cells[12]. Over-expressed keratin may be of diagnostic and prognostic interest to tumor patients in some tumor tissues.

CK17 is a keratin encoded by more than 30 different genes, expressed within the cytoplasm ,capable of reducing expression about the protein p27 cancer suppressor, so having a regulatory effect on the cycle of different tumor cells[13]. CK17 was not found to be expressed in normal cervical tissue but in squamous epithelium of cervical squamous cell carcinoma, which are used being the indication to analysis and prediction of cervical cancer[14]. Nazarian et al have found that CK17 within most anal invasive squamous cell carcinoma (SCC) is diffusely positive, but is typically negative within the anal squamous intraepithelial neoplasm (AIN) or only positive in the lesion surface. CK17 are used as an infiltration of differentials. SCC and non-invasive markers of AIN lesions[15]. Wang et al found that the level of expression of CK17 within oral squamous cell carcinoma was significantly higher than that of normal oral tissues and the manifestation had been significantly compared to the prognosis of oral adenosquamous carcinoma in oral carcinoma patients[16]. Another study found that expression of CK17 in a mixture of ovarian cancer cells had increased significantly. Silent expression of CK17 may meaningfully prevent cell propagation , immigration and ovarian cancer cell conquest, and encourage apoptosis of cells. Furthermore, CK17 is also expressed positively in triple-negative breast cancer which affects the patient's survival cycle[17].

The conducted research explores the appearance of CK17 had first exposed through immunohistochemical staining in 72 gastrointestinal tumor nerves in subsequent regular nerves. It is observed that the positive rate of manifestation of CK17 in gastric cancer tissue was 65.28 percent, which was substantially higher than that of adjacent normal tissue identified by RT-PCR, which was 9.72 per cent. The findings were consistent with staining of the immunohistochemistry, suggesting that CK17 expressed in gastric cancer tissue was up-regulated. To verify the clinical significance of appearance of CK17, we first classified samples of gastric cancer tissue as high expression and small appearance cluster founded at appearance of CK17 and investigated the correlation between expression of CK17 and clinicalopathological parameters for diseased persons through gastrointestinal growth. Results suggested that extraction of CK17 had been carefully linked to the patient's involvement of TNM staging and lymph node metastases. Expression of CK17 in tumor tissue within diseased persons concerning phase I and phase II TNM gastric cancer was substantially lower than within diseased persons concerning phase III and phase IV TNM. It is argued , the expression CK17 slowly improved with

an increase in the TNM level. Within the elevated-representation unit CK17, risk of lymph node metastases had been greater compared to short-communication unit , indicating that CK17 could promote gastric cancer cell metastases through unknown molecular mechanisms. We determined the Kaplan-Meier survival curve based on the prognostic data of the diseased persons for assessment the influence of CK17 at the prognosis of diseased persons along with gastrointestinal tumor. Tests showed that patients with low CK17 expression had substantially greater OS and DFS than those with high CK17 expression. Hence patients with elevated CK17 expression typically have a shorter survival time, indicating that expression of CK17 affects the prognosis of patients with gastric cancer. We performed a single-factor and multi-factor study of gastric cancer based on the patients' OS to further validate the clinical importance of CK17 in the prognosis of patients with gastric cancer. outcomes showed that expression of CK17, performance of TNM and metastasizing of the lymph node were independent risk factors to the whole existence of diseased persons along with gastrointestinal tumor. Additionally, CK17 has been shown to be a highly strong indicator for diseased persons along with gastrointestinal tumor.

For check the influence of CK17 on the biological role of gastrointestinal malignancy cells, it is said that levels of appearance of CK17 mRNA and protein within gastrointestinal growth cell lines had been meaningfully developed compared to the usual gastrointestinal mucosal cells, and the rates of expression of CK17 mRNA were also different within diverse gastrointestinal tumor cell lines. For subsequent functional experiments SGC-7901 with the highest expression level of CK17 was chosen. Small RNA interference (siRNA), gene cloning and other molecular tools are performed a vital part within the study of the mechanism of gastric cancer within current days through the enhancement of molecular biology techniques. SiRNA is a dual-stranded RNA chemically modified, with high efficiency of interference. Once the siRNA reaches the cells through a transient transfection, Dicer can recognize it and bind it to the intracellular RISC. It plays a role in homologous mRNA degradation, and thus silences the expression of a particular gene[18]. Therefore the expression CK17 in SGC-7901 cells was silenced by lentivirus for the sake of research the influence of CK17 at the biological role of gastric cancer. The western blotting tests revealed a substantial reduction within the manifestation of CK17 after silencing. That group's cell proliferation potential was measured using the CCK-8 method and assay on the formation of cell colony. The results showed that after expression of CK17 SCK-7901 was silenced, although development of cell proliferation had been considerably smaller compared to adverse power unit. CK17 is indicated being capable to facilitate the spread of gastric cancer cells.

Clinically, tumor and metastasis invasion is a significant problem within the treatment of gastrointestinal tumor and is also an essential element within tumor recurrence in patients with tumours. Transwell was used to detect cell resettlement and attack of gastrointestinal tumor cells to assess the impact of CK17 on the gastrointestinal tumor cell resettlement and aggression. conclusions showed that, after silencing CK17, the amount of migratory and aggressive cells decreased considerably, suggesting that CK17 can facilitate the movement and aggression of gastric cancer cells. Epithelial-mesenchymal shift (EMT) refers to process of epithelial cell transformation into stoma cells, and is closely related to embryonic growth, wound healing, tumor invasion, and metastasis[19]. The research works conducted earlier explored that EMT develops in cells with gastrointestinal tumor and works the key part regarding cell penetration and movement [20~21]. We used Western blots for discovery of the EMT-correlated protein expression during gastrointestinal tumor cells. Results showed that CK17's down-regulation substantially inhibited the expression of N-cadherin, Vimentin and some-catenin proteins and enhanced representation of E-cadherin. CK17 is indicated to be able to support the gastric cancer cell EMT process.

In conclusion, CK17 is highly regulated and works the vital part as oncogenes regarding gastric cancer. level of expression is closely connected to disorder development and the prognosis of gastrointestinal tumor diseased persons. CK17 can boost cell production , immigration and incursion of gastrointestinal tumor cells by promoting gastrointestinal tumor cell EMT cycle, and inhibiting apoptosis of gastric cancer cells. It is proposed that CK17 could be used as a possible objective for treating gastrointestinal tumor and as a prognostic biomarker.

Conflict of interest

No conflict of interest is proclaimed by the researchers.

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Table 1 Connection between CK17 and clinical characteristics of Gastric cancer patients

Characteristic	CK17 expression		p-value
	Low(n=25)	High(n=47)	
Gender			0.736
Male	16	31	
Female	9	16	
Age(years)			0.527
<50	7	12	
≥50	18	35	
Differentiation			0.412
Good/Moderate	10	19	
Poor	15	28	
Tumor size (cm)			0.121
≥4	16	30	
< 4	9	17	
TNM staging			0.002*
I~II	14	18	
III~IV	11	29	
Lymph node metastasis			0.012*
Yes	10	36	
No	15	11	

p-value had been gained by Pearson chi-square test.
*Statistically meaningful ($p<0.05$).

Table 2 Univariate and Multivariate assessment of clinicopathological issues for OS

Variable	Univariate Cox's Regression analysis		Multivariate Cox's Regression analysis	
	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
CK17 expression (high vs. low)	1.415 (0.718-2.156)	0.012	1.982 (0.726-2.544)	0.005*
Sex (male vs. female)	2.472 (1.258-3.229)	0.318	-	-
Age (<55 years vs. ≥55 years)	2.185 (1.122-3.068)	0.082	-	-
Tumor size (≥4cm vs. < 4)	0.895 (0.518-1.622)	0.925	-	-
TNM staging(I~II vs. III~IV)	1.321 (0.852-2.017)	0.021	1.786 (0.864-2.925)	0.012*
Differentiation(poor vs. good/moderate)	1.329 (0.635-2.271)	0.622	-	-
Lymph node metastasis(Yes vs. No)	1.618 (1.117-2.274)	0.006	1.276 (1.069-2.538)	0.002*

p-value was acquired by Cox proportional hazards degeneration.
*Statistically significant (p<0.05).

Figure legends
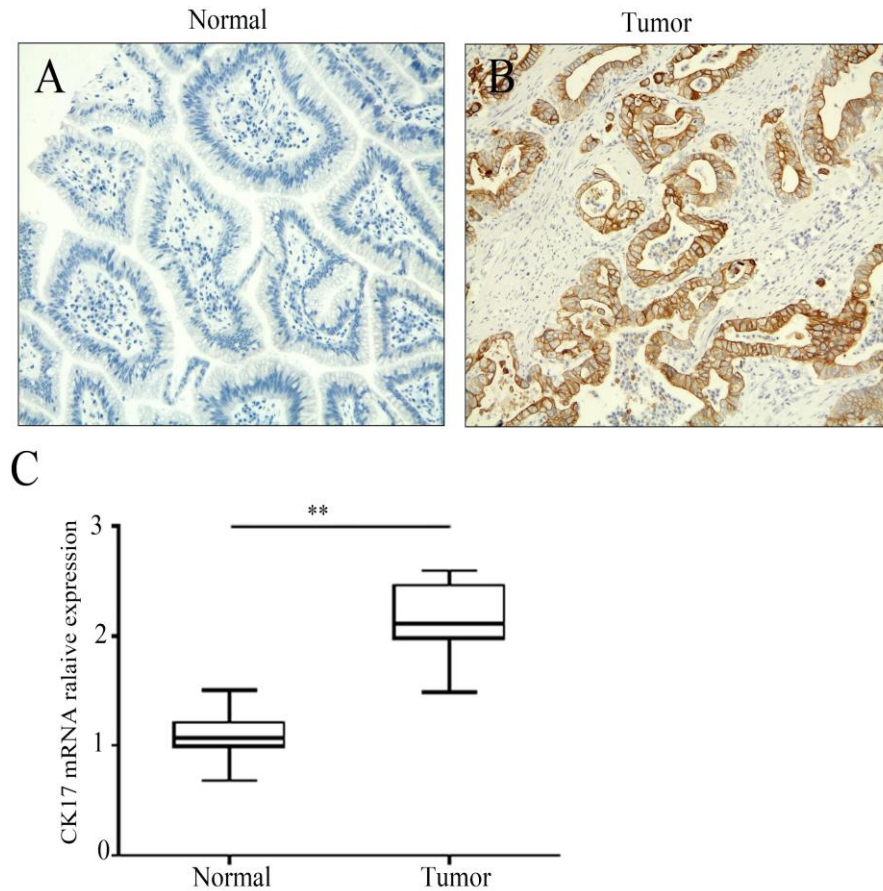
Fig 1. Expression of CK17 in gastric cancer tissues and adjacent normal tissues
(A) CK17 expression in gastric cancer tissues; (B) CK17 expression in adjacent normal tissues; (C) CK17 mRNA expression within gastrointestinal tumor tissues and adjacent normal tissues;
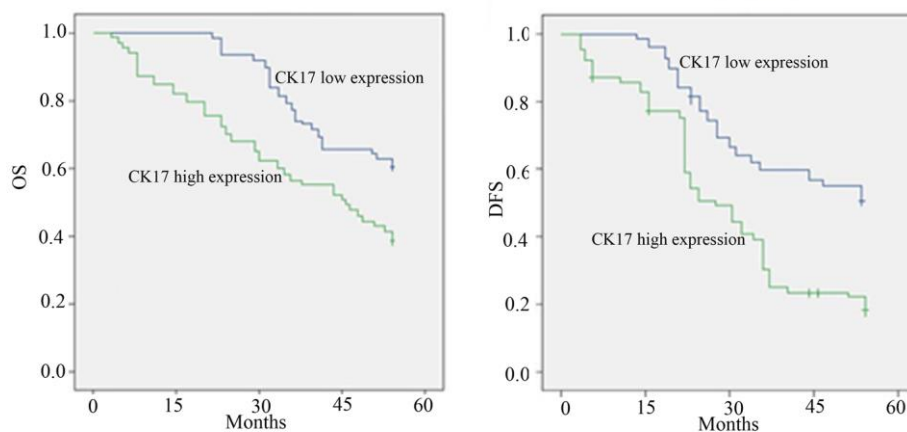

Fig 2. Comparison of survival period of gastric cancer patients with high expression and low expression level of CK17
(A) Total life cycle of gastric cancer diseased persons relating elevated communication and minimal representation level of CK17; (B) Disease-free survival of gastric cancer patients with high expression and low expression level of CK17.

(A)   (B)
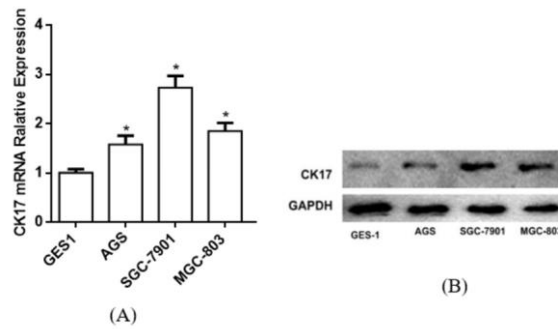
Fig 3. Protein and mRNA level of CK17 in various gastric cancer cell lines
(A) mRNA level of CK17 in various cell lines gastric cancer; (B) Protein level of CK17 regarding several cell lines gastric cancer
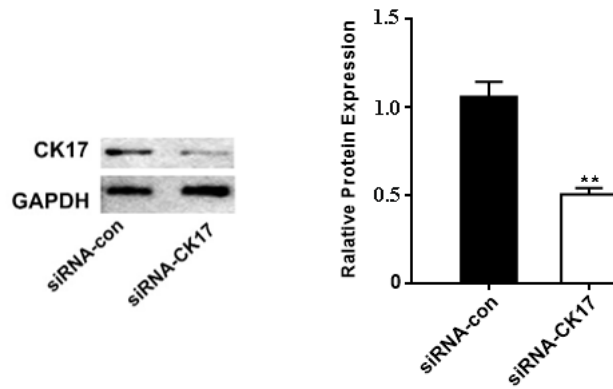

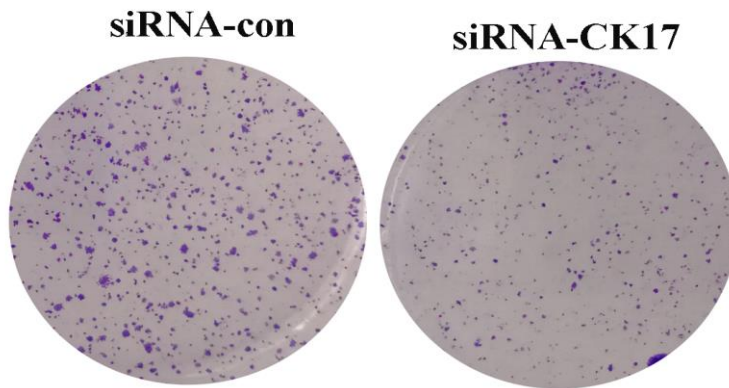Fig.4 Protein level of CK17 in various cell lines silencing of CK17

Fig.5 Comparison of cell viability of gastric cancerous cell with CK17 silencing
(A) EDU was used to detect the cell viability in gastric cancer cells; (B) cell colony formation assay in detection of the cell proliferation capacity in gastric cancer cells.
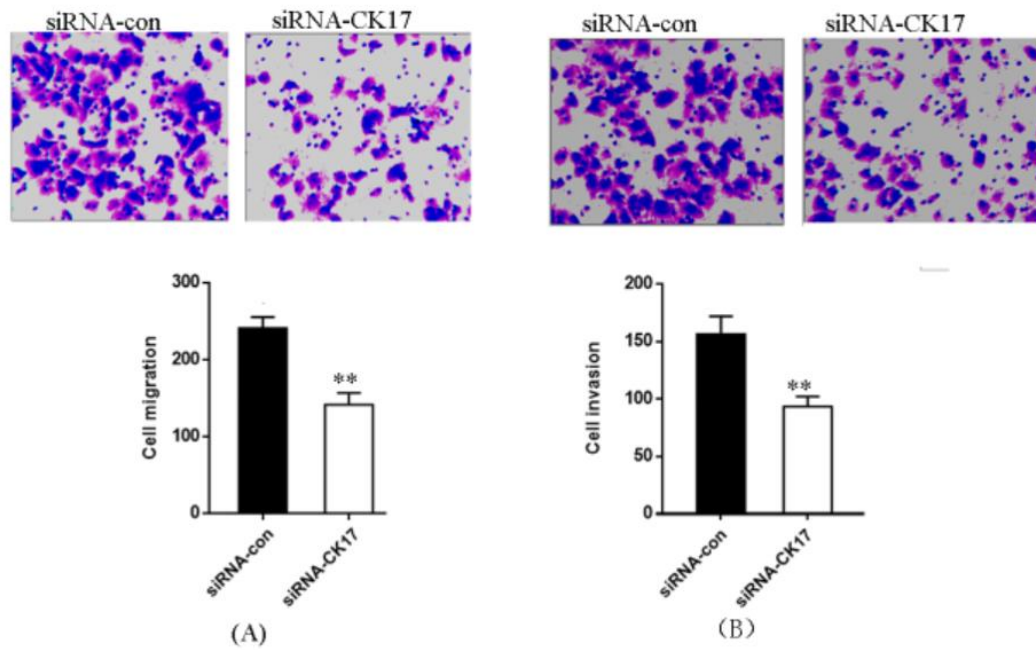
Fig 6. Cell migration and invasion capacity of gastric cancer cells
(A) Comparison of cell migration capacity of SGC-7901 with CK17 silencing; (B) Comparison of cell invasion capacity of SGC-7901 cells with CK17 silencing and overexpression.
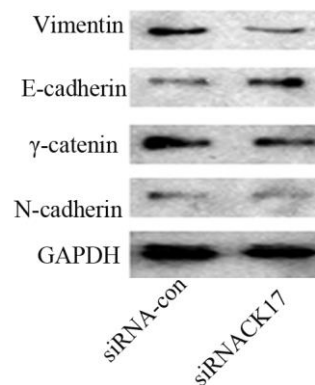

Fig 7. CK17 promotes EMT process of gastric cancer cells